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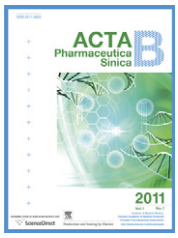

REVIEW

Role of microRNAs in cardiac hypertrophy, myocardial fibrosis and heart failure ☆

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Abstract MicroRNAs (miRNAs) are endogenous small non-coding RNA molecules that post-transcriptionally regulate gene expression. MiRNA expression and function in heart disease remain to be determined but modulation of miRNA expression *in vivo* has revealed that miRNAs play an important role in controlling heart function and structure. In fact, abnormal expression of miRNAs may initiate and contribute to the progress of heart disease. Here, we summarize the literature relating to the involvement of miRNAs in cardiac hypertrophy, myocardial fibrosis and heart failure.

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1. Introduction

MicroRNAs (miRNAs) are small 22–24 nucleotide non-coding RNA molecules that regulate gene expression by hybridization to 3′ untranslated regions (3′ UTRs) of target messenger RNAs (mRNAs) resulting in their degradation or translational inhibition[1]. Collectively more than 4000 miRNA sequences exist in a wide range of species of which more than 700 are encoded by the human genome[2]. Of the latter, some 150–200 are expressed in the heart where they are dynamically regulated in response to cardiac disease[3].

MiRNAs constitute a particularly abundant and fundamental class of regulators of gene expression. Apart from their involvement in developmental aspects of higher organisms, they are now implicated in an increasing number of physiological processes. Initially, miRNAs were shown to play important roles in plant biology[4], viral diseases[5], developmental processes[6] and cancer[7]. More recently, several studies have demonstrated their importance in the regulation of cardiac development[8] and various cardiovascular diseases such as myocardial infarction[9], cardiac hypertrophy[10] and heart failure[11].

The heart responds to injury and hemodynamic overload by promoting myocyte hypertrophy, reexpressing a fetal gene program and remodeling the extracellular matrix. Specific miRNAs are disregulated in the diseased heart and, in mice, up- and down-regulation of miRNAs have been found to be necessary and sufficient to explain diverse heart diseases[12]. Thus, normalizing miRNA expression in the heart represents a new approach to the pharmacotherapy of heart disease[13–15].

In this paper, we review miRNA biology in general and the literature relating to the mechanisms of miRNA action in cardiac hypertrophy, myocardial fibrosis and heart failure. Understanding how miRNAs regulate not only single genes but the whole gene network has enormous therapeutic implications.

2. MiRNA biogenesis and biological function

MiRNAs are encoded in the genomes of plants and animals. They are initially transcribed by RNA polymerase II or, in a few cases, RNA polymerase III as long primary miRNAs (pri-miRNAs) containing a 65-nucleotide stem loop[16]. Pri-miRNAs fold into hairpin structures containing imperfectly base-paired stems and are processed by the endonuclease Drosha and its cofactor DiGeorge syndrome critical region 8 (dgcr8) into 60–100 nt hairpins known as pre-miRNAs[17,18]. Pre-miRNAs are then exported to the cytoplasm by Exportin-5 and subsequently processed by the cytoplasmic RNaseIII, Dicer, which also resides in a multiprotein complex[19].

Cleavage of the pre-miRNA by Dicer produces mature miRNAs, which are incorporated into the RNA-induced silencing complex (RISC). This then recognizes specific targets and induces post-transcriptional gene silencing through binding of the mature miRNA to the 3′ untranslated region of its target mRNA through exact and partial complementarity[20]. Depending on the overall degree of complementarity, gene silencing occurs through either inhibition of translation or degradation of its target mRNAs. Interestingly, in rare cases, miRNAs have also been reported to stimulate mRNA translation[21], a finding which has sparked controversy and led to demands for more research into the mechanisms by which miRNAs regulate gene expression[22].

3. MiRNAs and cardiac hypertrophy

Cardiac hypertrophy is an important compensatory mechanism of the heart in response to diverse pathophysiological stimuli. Initially, the response aims to normalize wall stress and preserve contractile performance but chronically it produces hypertrophy and may eventually lead to heart failure. Although various pathways provide coordinated control of the hypertrophic process, little is known about their underlying molecular mechanisms. Exposure of the heart to some stressors may lead to cardiac remodeling with a change in the gene expression profile and a detrimental outcome. The involvement of miRNAs in this pathological process is now recognized and increasing evidence shows that miRNAs are key modulators of cardiovascular development and function and the process of cardiac hypertrophy.

Number of miRNAs are found to be regulated during cardiac hypertrophy and two, miR-1 and miR-133, play a key role in inhibiting it. MiR-1 and miR-133 are included in the same bicistronic unit and are specifically expressed in skeletal muscle and cardiac myocytes[8]. Embryonic overexpression of miR-1 *in vivo* results in thin-walled ventricles, whereas miR-1 knockout mice display chambers with thickened walls[23]. MiR-1 is down-regulated at the onset of pressure overload on the heart that appears to be sufficient to account for the changes in gene expression underlying the initiation and progression of cardiac hypertrophy[24]. MiR-1 down-regulates calcium–calmodulin signaling through the calcineurin/NFAT pathway and negatively regulates the expression of Mef2a and Gata4 to inhibit cardiomyocyte growth[25]. The cytoskeleton regulatory protein, twinfilin-1, is a novel target of miR-1 and reduction of miR-1 by hypertrophic stimuli up-regulates twinfilin-1 which, in turn, evokes hypertrophy through regulation of the cardiac cytoskeleton[26].

MiR-133 has a critical role in determining cardiomyocyte hypertrophy; its overexpression inhibits hypertrophy whereas its suppression induces hypertrophy both *in vitro* and *in vivo*[10]. Recently, Dong et al.[27] found miR-133 expression was down-regulated, and calcineurin activity enhanced in both *in vivo* and *in vitro* models of cardiac hypertrophy[27]. In addition, they found that using cyclosporine A to inhibit calcineurin prevented the down-regulation of miR-133 in cardiac hypertrophy. These results indicate that miR-133 and calcineurin are reciprocally repressed in cardiac hypertrophy. Moreover, another study indicated that miR-133a plays a role in diabetes-induced cardiomyocyte hypertrophy; down-regulation alters gene expression and mediates glucose-induced cardiomyocyte hypertrophy through SGK1 and IGFR1[28]. However, van Rooij et al.[29] found that miR-133 did not produce any of the morphological changes in cardiomyocytes associated with hypertrophic growth[29].

Several other miRNAs have been found to be pro-hypertrophic including miR-208, 21, 18b, 195, 199, 23, 24, 27 and 9. MiR-208 is cardiac specific and is shown to be necessary for cardiomyocyte hypertrophy, fibrosis and expression of β-MHC in response to stress and hypothyroidism[30]. Mice subjected to targeted deletion of miR-208 failed to undergo stress-induced cardiac remodeling, hypertrophic growth and

β-MHC up-regulation; whereas, overexpression of miR-208 was shown to induce cardiac hypertrophy. During cardiac hypertrophy, a reduction in the expression of α-MHC resulted in diminished transcription of miR-208 and subsequently to loss of its negative effects on thyroid hormone receptor associated protein 1 (THRAP1) and to a blunted response to pressure overload.

It is interesting to speculate on whether modulation of miR-208 *in vivo* is a potentially useful therapeutic strategy in cardiac hypertrophy. In support of this, Callis et al.[31] reported that overexpression of miR-208a in mouse heart was sufficient to induce hypertrophic growth resulting in pronounced repression of the miR-208 regulatory targets, THRAP1 and myostatin, the negative regulators of muscle growth and hypertrophy[31].

Tatsuguchi et al.[32] reported that inhibition of endogenous miR-21 or miR-18b augmented hypertrophic growth whereas introduction of functional miR-21 or miR-18b into cardiomyocytes repressed it[32]. Despite this, the effect of miR-21 on myocyte hypertrophy remains controversial. Cheng et al.[33] found that miR-21 was strikingly up-regulated both in hypertrophic mouse hearts and cultured neonatal hypertrophic cardiomyocytes and that modulating miR-21 expression via antisense-mediated depletion produced a significantly negative effect on cardiomyocyte hypertrophy[33]. Recently, Patrick et al.[34] reported that miR-21 was not essential for pathological cardiac remodeling based on the fact that miR-21-null mice did not display improved tolerance to a variety of cardiac stressors compared to their wild-type littermates and that inhibition of miR-21 failed to block stress-induced cardiac remodeling[34].

Overexpression of miR-195 during cardiac hypertrophy results in pathological cardiac growth and heart failure in transgenic mice[29]. MiR-199a is predominantly expressed in cardiomyocytes where it maintains cell size and may play a role in the regulation of cardiac hypertrophy. It is also confirmed to target hypoxia-inducible factor 1 alpha[35]. Previously, it was shown that miR-23a, 27a and 24-2 are up-regulated during cardiac hypertrophy[36] and that knockdown of miR-23a attenuates hypertrophy[37]. Finally, miR-9 appears to regulate cardiac hypertrophy by suppressing myocardin expression[38].

4. MiRNAs and cardiac fibrosis

Cardiac fibrosis is an established morphological feature of structural myocardial remodeling that occurs in several cardiac diseases including myocardial infarction, dilated hypertrophic cardiomyopathies and heart failure. The cellular basis of fibrosis is the adverse accumulation of collagens and other extracellular matrix (ECM) proteins that increase the risk for adverse cardiovascular events such as ventricular dysfunction and arrhythmias. The etiology of the fibrogenic cardiac phenotype is still being elucidated but a small number of studies have already demonstrated that altered expression of several miRNAs in myocardial fibrosis is associated with ischemia or mechanical overload.

MiR-29 acts as a regulator of cardiac fibrosis and represents a potential therapeutic target for tissue fibrosis in general[39]. The miR-29 family targets a series of mRNAs that encode proteins involved in fibrosis, including multiple collagens, fibrillins and elastin. Down-regulation of miR-29 is therefore predicted to repress the expression of some mRNAs and enhance the fibrotic response. Indeed, such down-regulation with anti-miRs *in vitro* and *in vivo* induces the expression of collagens whereas overexpression of miR-29 in fibroblasts reduces it[39]. Down-regulation of miR-149 and up-regulation of miR-21, 214 and 223 were shown to accompany down-regulation of miR-29[39], but the functional consequences of these changes are unknown.

MiR-21 contributes to myocardial remodeling through regulating the ERK-MAP kinase signaling pathway in cardiac fibroblasts during cardiac ischemia/reperfusion[40] or in the later stages of heart failure[41]. *In vivo* silencing of miR-21 by a specific antagomir suppresses pathological ERK-MAP kinase signaling and prevents cardiac dysfunction in a mouse pressure-overload-induced disease model. MiR-21 regulates fibroblast survival and growth factor secretion that ultimately control the extent of interstitial fibrosis and cardiac hypertrophy[41]. Overall, these findings indicate that miR-21, like miR-29, contributes to myocardial remodeling by affecting cardiac fibroblasts. Down-regulation of miR-21 could therefore be a beneficial approach to block fibroblast proliferation in heart disease and thereby inhibit secondary cardiac remodeling.

The potential importance of miR-133 in cardiac fibrosis has been underlined by reports that miR-133-a1 and miR-133-a2 knockout mice develop severe fibrosis and heart failure[42] and that miR-133 and miR-590 are down-regulated in a canine model of nicotine-induced atrial interstitial fibrosis[43,44]. Duisters et al.[45] reported that knockdown of miR-133 or miR-30 increased the level of connective tissue growth factor (CTGF) and that overexpression of miR-133 or miR-30 decreased CTGF levels accompanied by decreased production of collagens[45]. They showed that CTGF was a direct target of these miRNAs because they directly interacted with the 3′ untranslated region of CTGF. Another report showed that transgenic expression of miR-133a prevented TAC-associated miR-133a down-regulation and improved myocardial fibrosis and diastolic function without affecting the extent of hypertrophy[46]. Interestingly, mechanistic studies in the canine model and in cultured atrial fibroblasts showed that the protective actions of miR-133 and miR-590 are mediated through targeting TGF-b1 and TGF-b receptor type II, respectively[44].

In addition to cardiac fibrosis, miRNAs are involved in the fibrotic process in other organs such as lung, kidney and liver[47]. However, much research remains to be done to identify which miRNAs have a direct role in the development of fibrosis, and which have altered expression secondary to cardiac fibrosis. Characterization of individual miRNAs or miRNA expression profiles that are specifically associated with myocardial fibrosis will hopefully allow the development of diagnostic tools and innovative therapies for fibrogenic cardiac diseases.

5. MiRNAs and heart failure

As stated earlier, the heart responds to cardiac injury or hemodynamic overload by activating a variety of intracellular signaling pathways that provoke myocyte hypertrophy, re-expression of embryonic genes and remodeling of the extracellular matrix[48]. When the heart transits from adaptive to maladaptive hypertrophy, heart failure occurs. The pathological mechanism of heart failure is a result of the

concomitant cross-talk between various deleterious and compensatory signaling pathways, the balance between which ultimately determines the pathological progression. Despite significant advances in the identification of genes and signaling pathways, the overall complexity of cardiac hypertrophic remodeling suggests the involvement of additional global regulatory signaling networks. A growing body of evidence suggests that some miRNAs are involved in the process of heart failure.

Cardiomyocyte-specific deletion of a gene or endonuclease, such as dgcr8 and Dicer, which are required for microRNA biogenesis and processing, leads to a dramatic fall in the level of mature miRNAs and to left ventricular malfunction progressing to a dilated cardiomyopathy and premature lethality[49,50]. Sucharov et al.[51] demonstrated that the expression of subsets of miRNAs was differentially regulated in different heart failure models[51]. They found that inhibition of miR-100, which was up-regulated in the failing heart, prevented β-adrenoceptor (β-AR) mediated down-regulation of the adult gene component of the fetal gene program. They also suggested that overexpression of miR-133b attenuated β-AR mediated changes in gene expression. van Rooij et al.[29] showed that overexpression of individual stress-inducible miRNAs was sufficient to induce hypertrophic growth in isolated cardiac myocytes and to provoke a dilated cardiomyopathy in transgenic mice, suggesting that individual miRNAs are sufficient to provoke heart failure[29]. In their other study[30], they found that deletion of miR-208, a cardiac-specific miRNA encoded by an intron in the gene that encodes the α-myosin chain, protected against cardiac myocyte hypertrophy, up-regulated β-myosin heavy chain and produced myocardial fibrosis in response to thyroid signaling and hemodynamic overload. They also showed that animals deficient in miR-208 did not ramp up cardiac expression of β-myosin heavy chain in response to thyroid signaling.

MiR-199b is a direct calcineurin/NFAT target gene the expression of which is increased in mouse and human heart failure. miR-199b targets nuclear NFAT kinase dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1a (Dyrk1a) in a process that constitutes a pathogenic feed forward mechanism affecting calcineurin-responsive gene expression[52]. *In vivo* inhibition of miR-199b by a specific antagomir normalized Dyrk1a expression, reduced nuclear NFAT activity and caused marked inhibition and even reversal of hypertrophy and fibrosis in mouse models of heart failure. Naga Prasad et al.[53] examined the alterations of miRNA expression in human heart failure samples in order to elucidate the global regulation of the signaling networks by a unique miRNA pattern in end-stage human heart failure[53]. They showed that eight miRNAs (miR-1, 214, 29b, 342, 7, 125, 378 and 181) were significantly altered in dilated cardiomyopathy compared with non-failing controls. They also identified two novel miRNAs (miR-7 and 378) that were down-regulated in end-stage heart failure. Tijsen et al.[54] identified a number of circulating miRNAs as putative biomarkers of heart failure and showed that miR423-5p in particular distinguished heart failure cases from non-heart failure cases[54].

Transfection of isolated adult rat cardiomyocytes with a set of fetal miRNAs (miR-21, 129 and 212) resulted in hypertrophic morphological changes in neonatal cardiomyocytes similar to those observed in the failing heart but transfection of any one of the set produced only minor effects[11]. However, many reports indicate that associations exist between single miRNAs and heart failure and indeed that the pathological changes in heart failure may require an alteration of the miRNA environment.

6. MiRNAs-based strategies for diagnostics and therapeutics

MiRNA expression has been shown to play a role in the growth, development, function and stress responsivenesses of the heart as well as in heart disease. The possibility of exploiting miRNAs as diagnostic markers or therapeutic tools has much to recommend it because of their specificity to their targets in a particular cellular pathway.

MiRNAs show different expression patterns in the normal and diseased heart and some data indicate that miRNA expression may represent an efficient diagnostic marker of heart disease[11,29]. Identification of abnormal miRNA levels in tissue or plasma could certainly aid in early diagnosis and, in fact, there is increasing evidence that circulating miRNAs in various body fluids are potential biomarkers of disease diagnosis[55–57]. For example, certain miRNAs have been proposed for screening colorectal cancer[58] and for monitoring pregnancy[59]. Recently, many miRNAs have been reported to be potential biomarkers for cardiovascular disease and, in particular, miR-208, miR-1 and miR-499 have been shown to indicate the diagnosis and progression of myocardial infarction[60–62]. In the case of miR-208, its plasma concentration increased in isoproterenol-induced myocardial injury and was well correlated with the plasma concentration of cTnI, the classical marker of myocardial injury[60]. For miR-1, the plasma level was significantly higher in patients with acute myocardial infarction (AMI) compared with non-AMI subjects and the level dropped to normal on discharge following medication[61]. For miR-499, plasma concentrations were significantly increased in patients with acute myocardial infarction but were below the detection limit in other patient groups[62].

Other circulating miRNAs have been proposed as sensitive and informative biomarkers for the diagnosis of heart failure. MiR423-5p is specifically enriched in the blood of heart failure patients but not in healthy people indicating that certain miRNAs are not only diagnostic predictors of heart failure but also a means of distinguishing clinical heart failure from other diseases[54]. The plasma concentration of miR-126 has also been proposed as a biomarker of heart failure[63]. Moreover, miRNAs are more sensitive than mRNAs in detecting end-stage heart failure[64]. These and other studies reveal the potential of miRNAs in assessing the risk of certain kinds of disease and in evaluating the efficacy of treatment. The development of miRNAs as biomarkers for cardiovascular diseases is still in its infancy and, no doubt, will remain an active field for many years yet.

Identification of specific miRNAs and target genes that contribute to adult cardiac pathology is likely to provide new therapeutic targets. In the classical course of drug discovery, it takes many years to identify such targets, devise and execute accurate screening methods, and eventually develop the molecules affecting those targets as therapeutic agents. However, miRNAs are not likely to require such a prolonged process since they can be efficiently manipulated to tune gene regulation and are more amenable to incorporation in drug

delivery systems because of their small size[65,66]. Furthermore, miRNAs are predicted to have multiple mRNA targets[67], some of which appear to work in concert to control a common pathway or biological function. However, this can also be seen as a major disadvantage because of its propensity to cause side effects. Although there are several approaches to change miRNA levels *in vivo* and *in vitro* as exemplified in numerous gain-of-function and loss-of-function studies, a detailed understanding of miRNA biology and function pertaining to the heart remains some way off.

Exogenous miRNAs, either synthetic or constructed in virus vectors, have been used to restore the decreased levels that accompany certain cardiovascular diseases and thereby retard the associated pathological process[24,41]. Generally, these miRNAs are double-stranded and have the same sequence as endogenous miRNAs. One technique to increase the cellular level of a specific miRNA is through the use of a miRNA mimic, which utilizes synthetic nucleic acids to bind to target mRNAs and elicit post-transcriptional regulatory effects. The double-stranded structure enables efficient recognition and loading into RISC to elicit miRNA action. This strategy can potentially repress the target gene at the post-transcriptional level with minimal effects on the mRNA level. These constructs are analogous to siRNA molecules and have been successfully utilized *in vitro*. Efficacy *in vivo* remains to be demonstrated.

Efficient reduction in the level of specific miRNAs associated with a particular disease should be therapeutically advantageous. Inhibiting miRNAs expression can be achieved using antisense inhibitor oligonucleotides (AMO) designed to fully complement their target miRNAs in order to degrade them. Besides being potentially therapeutic, the application of AMOs is a popular method for studying miRNA function. Furthermore, antagomirs based on cholesterol conjugated oligonucleotides have been used to facilitate cellular uptake and resolve the delivery problem faced by AMOs. Antagomirs which target a specific miRNA or disrupt the binding between an miRNA and its target represent a potentially effective way to inactivate pathological miRNAs. The first successful mammalian *in vivo* study using such an antagomir aimed to inhibit a liver-specific miR-122[66]. Further studies have since attempted to modify cellular uptake using high-density lipoproteins[68], 2′-*O*-methoxyethyl phosphorothioate[65], and locked-nucleic-acid-modified oligonucleotides[69] that were subsequently tested in non-human primates[70]. With respect to the heart, *in vivo* inhibition of miR-133 and miR-29 with antagomirs in mice has implicated their participation in cardiac hypertrophy and cardiac fibrosis, respectively[10,39]. Antagomirs are powerful tools to silence specific miRNAs *in vivo* and may represent a therapeutic strategy for silencing miRNAs in disease[66].

Another way to interfere with miRNA–mRNA interactions is through competitive inhibition of miRNA using "miRNA sponges". These can be expressed in cells as transcripts of strong promoters containing multiple, tandem binding sites to a related miRNA[71]. Comparable to sponges, miRNA erasers use only two copies of a perfectly complementary antisense sequence of a miRNA[72]. Recently, this approach has been tested in Drosophila in an attempt to understand the factors that contribute to the spatiotemporal specificity of miRNAs[73].

Research into the role of miRNAs in human heart disease holds great promise for future therapeutic applications. However, developing miRNAs into therapeutic agents faces significant challenges associated with their drug delivery and duration of action. Although local delivery to the heart through direct injection or via a catheter or coated stent would avoid these problems, its clinical feasibility remains to be determined.

7. Conclusions

MiRNAs have emerged as a novel class of key regulators in a variety of cardiovascular diseases including cardiac hypertrophy, fibrosis and heart failure. We summarize the current understanding of microRNAs in cardiac hypertrophy, myocardial fibrosis and heart failure in Fig. 1. MicroRNAs represent potential pharmacological targets since down- or up-regulation of a particular miRNA is enough to cause a specific cardiovascular disease and correcting their aberrant expression can reverse the pathological process. Ongoing efforts to identify the targets of specific miRNAs involved in

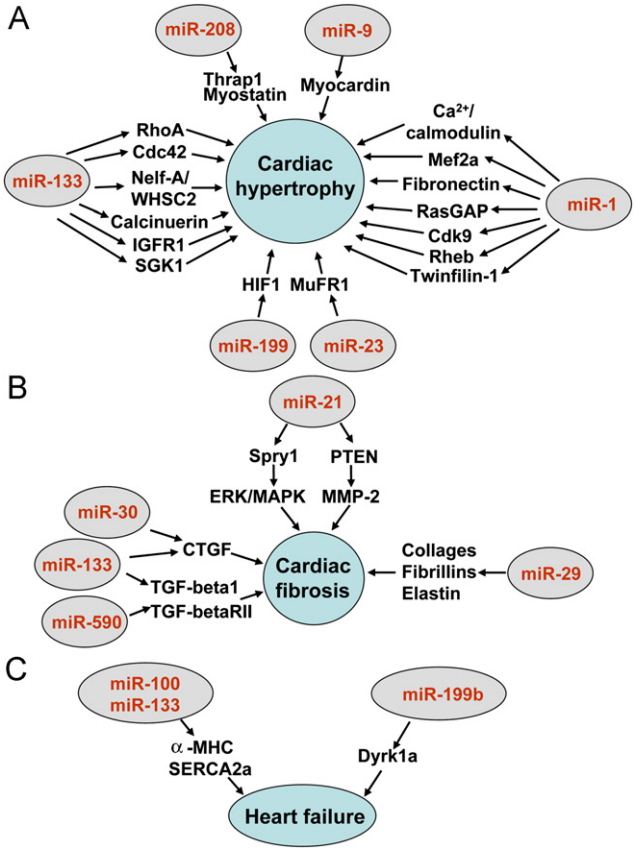

Figure 1 Current understanding of the role of microRNAs in cardiac hypertrophy, myocardial fibrosis and heart failure. Thrap1: thyroid hormone receptor associated protein 1; Cdc42: cell division cycle 42; Nelf-A: negative elongation factor A; WHSC2: Wolf–Hirschhorn syndrome candidate 2; IGFR1: insulin-like growth factor receptor 1; SGK1: serum- and glucose-regulated kinase; RasGAP: Ras GTPase-activating protein; Cdk9: cyclin-dependent kinase 9; Rheb: Ras homolog enriched in brain; MuFR1: the muscle specific ring finger protein 1; HIF1: hypoxia-inducible factor 1; CTGF: connective tissue growth factor; PTEN: tensin homolog; MMP-2: matrix metalloprotease-2; Spry1: sprouty homolog 1; α-MHC: alpha-myosin heavy chain; Dyrk1a: dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1a.

the cardiovascular system will increase our understanding of the molecular mechanisms underlying disease processes. However, the fact that a single miRNA can control hundreds of distinct target genes and potentially affect many cellular pathways means that achieving a full understanding of miRNA-mediated molecular networks in the heart is a significant challenge. Thus the routine application of miRNAs in the clinic remains a rather distant goal.

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